

FIG. 1a

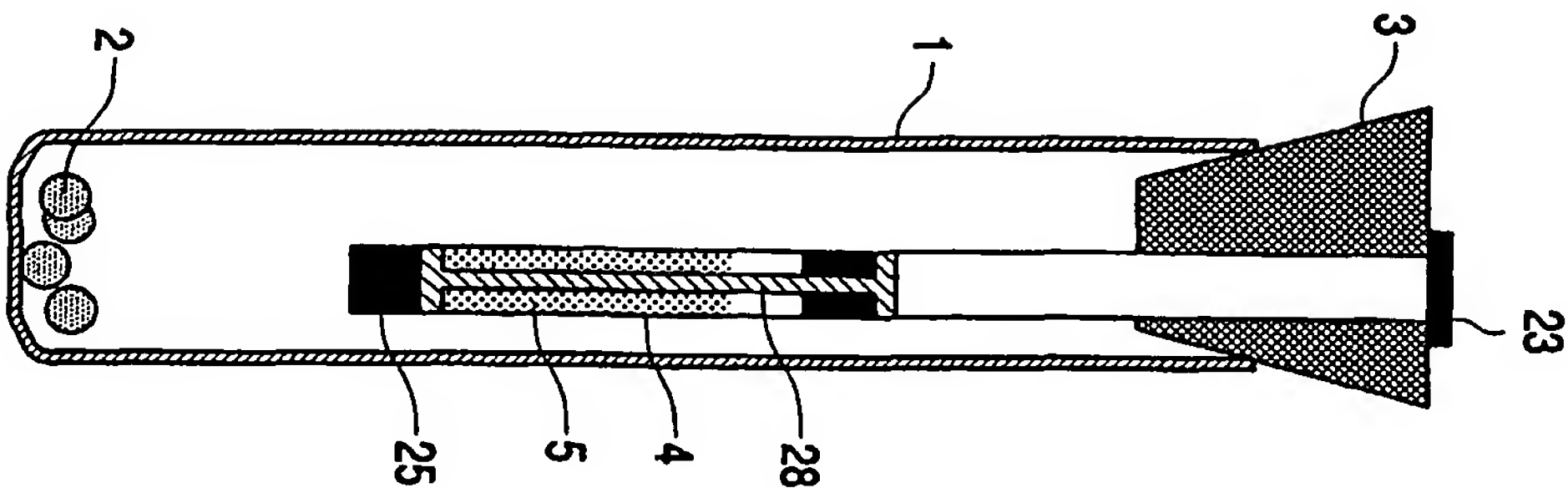


FIG. 1b

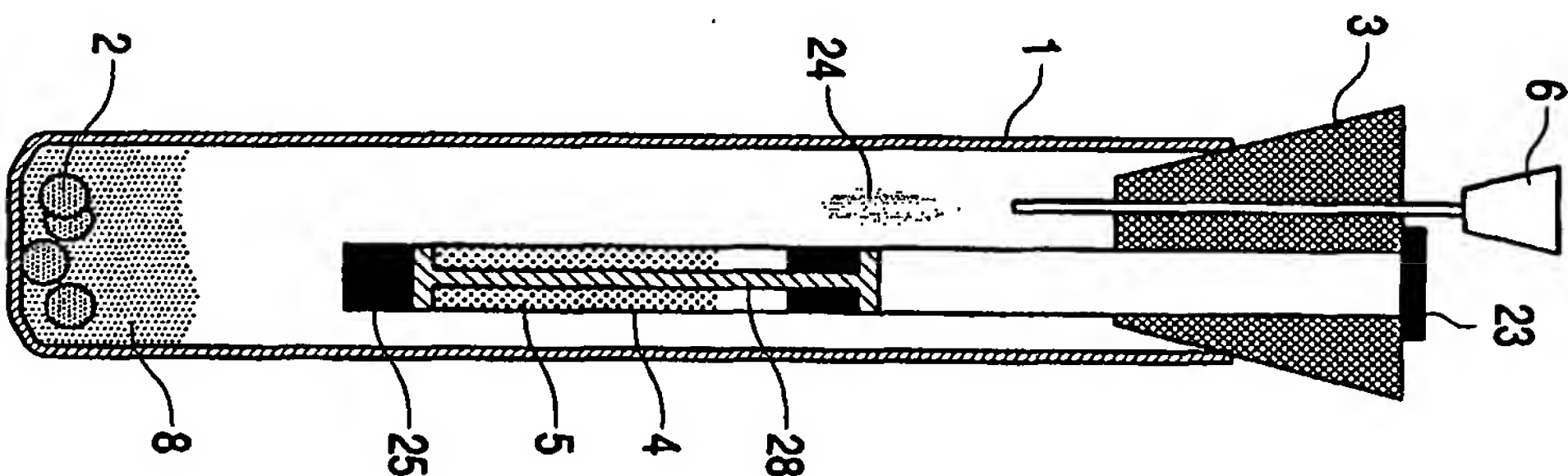


FIG. 1c

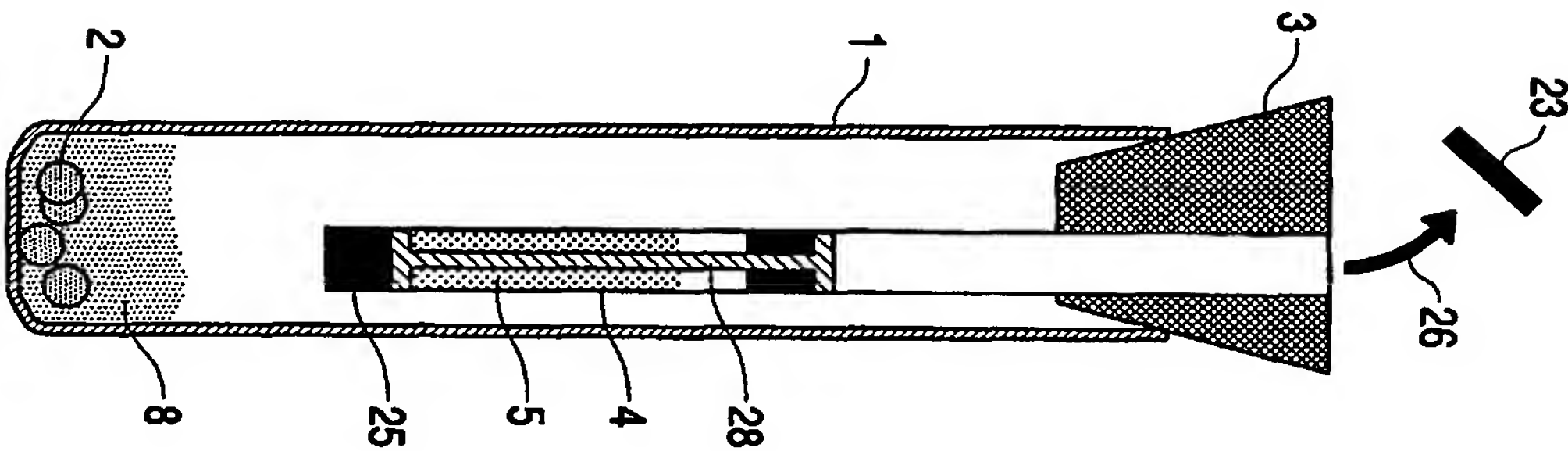
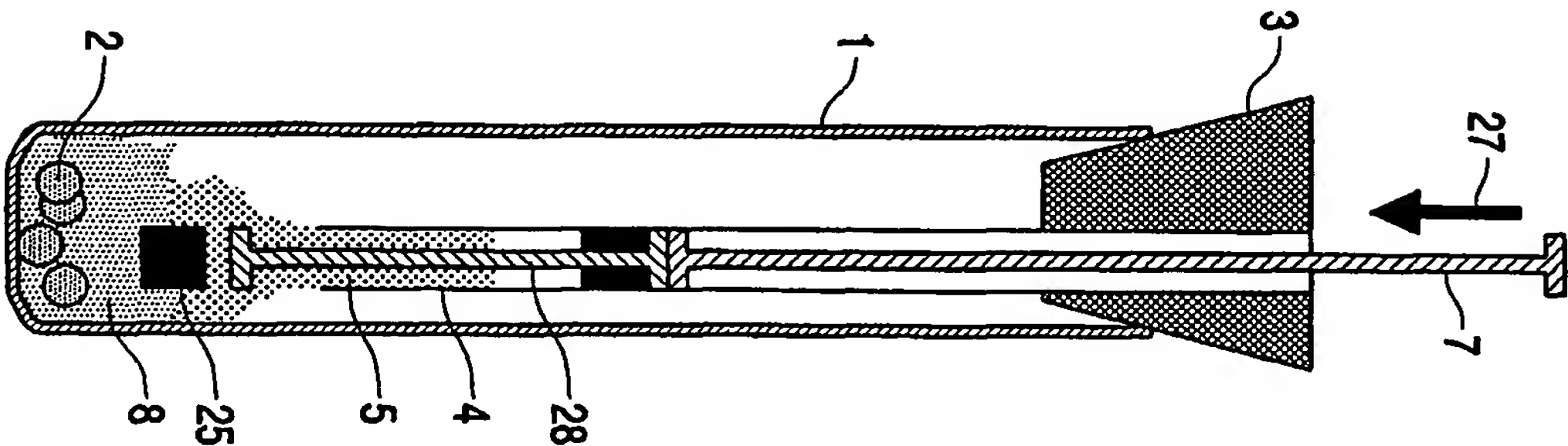


FIG. 1d



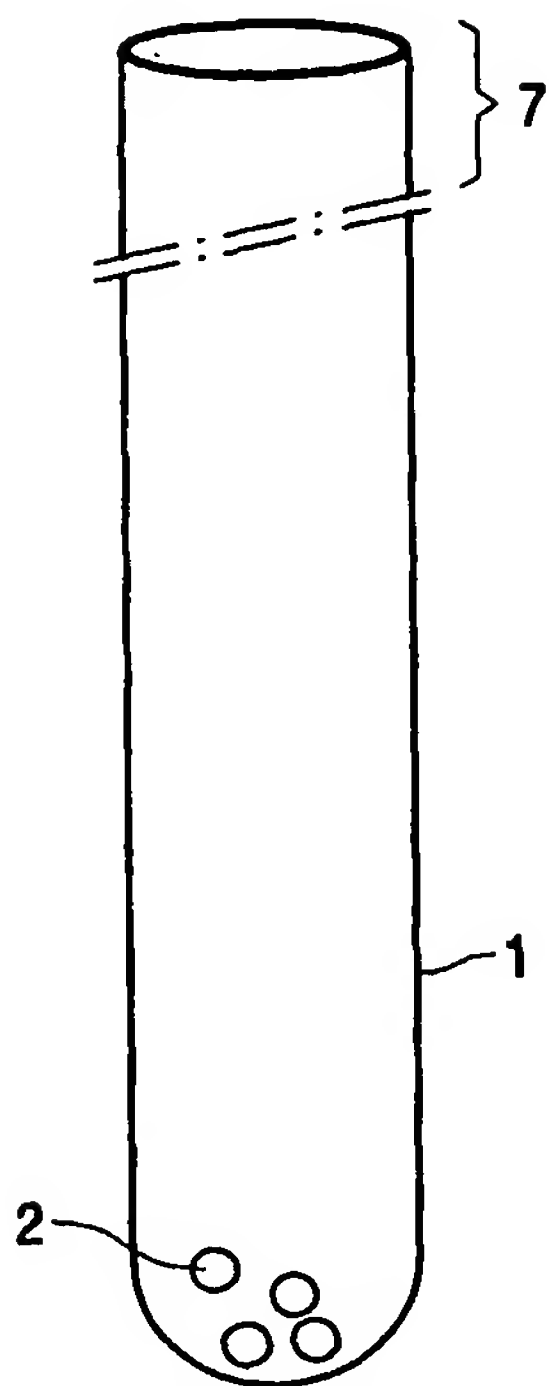


FIG. 2

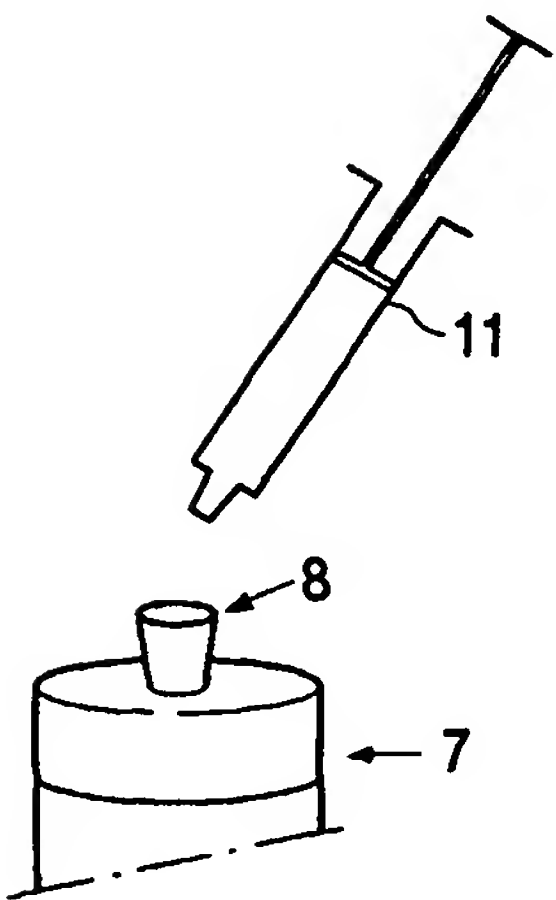


FIG. 3

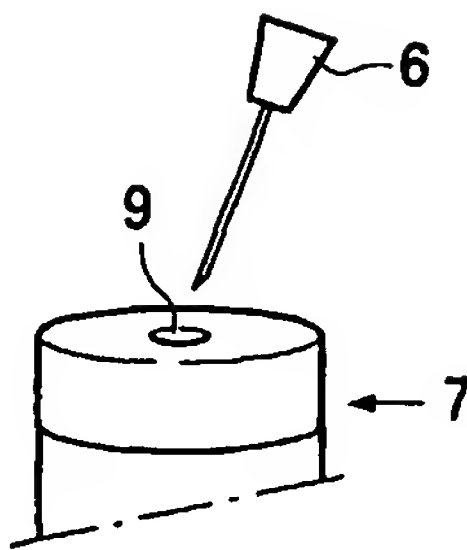


FIG. 4

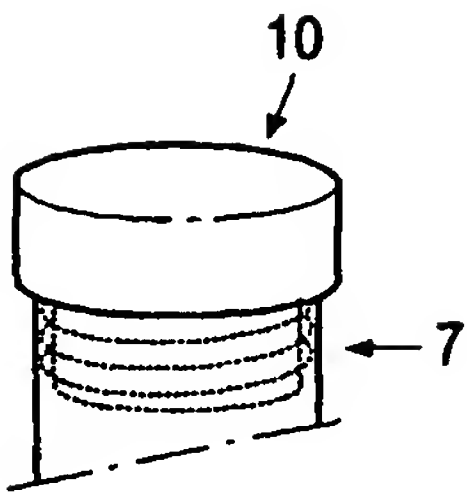


FIG. 5

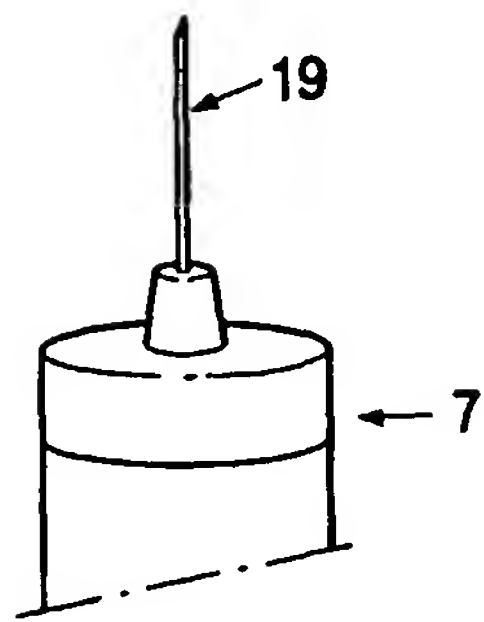


FIG. 6

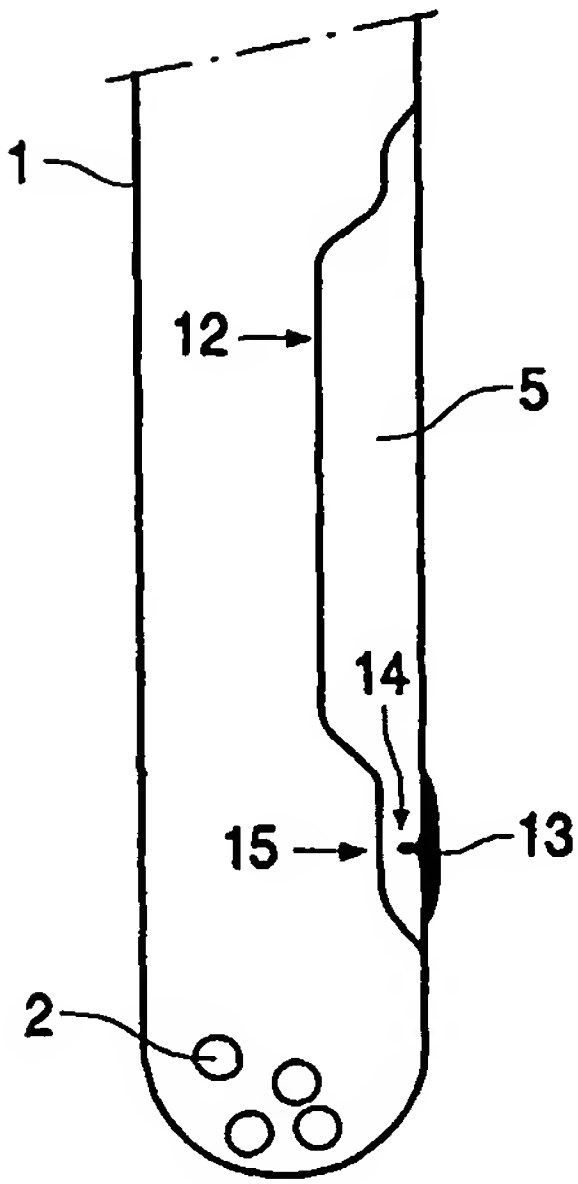


FIG. 7

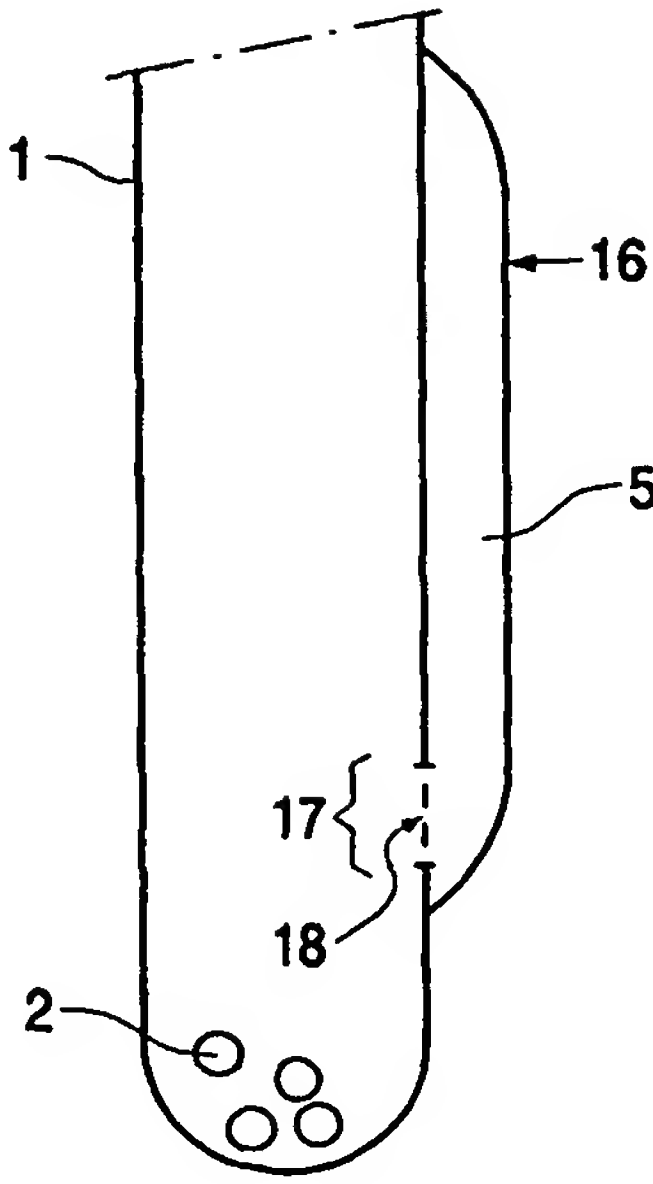


FIG. 8

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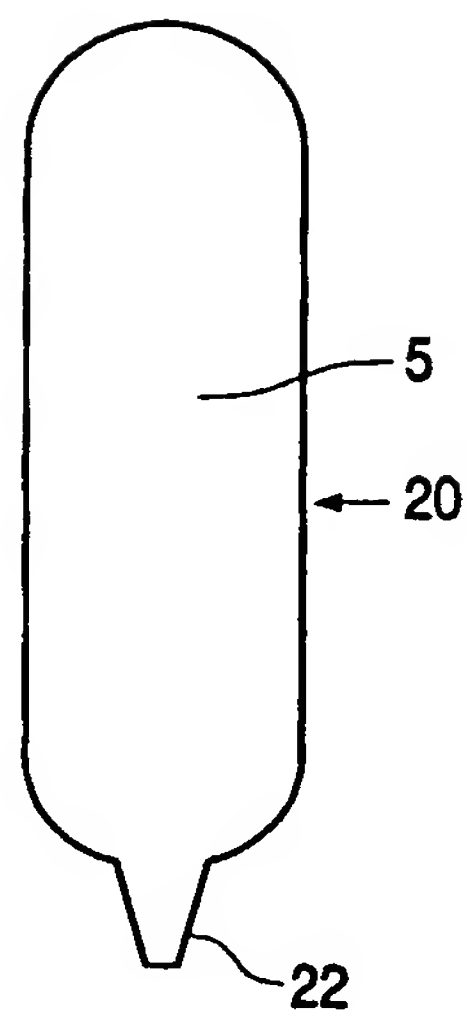


FIG. 9

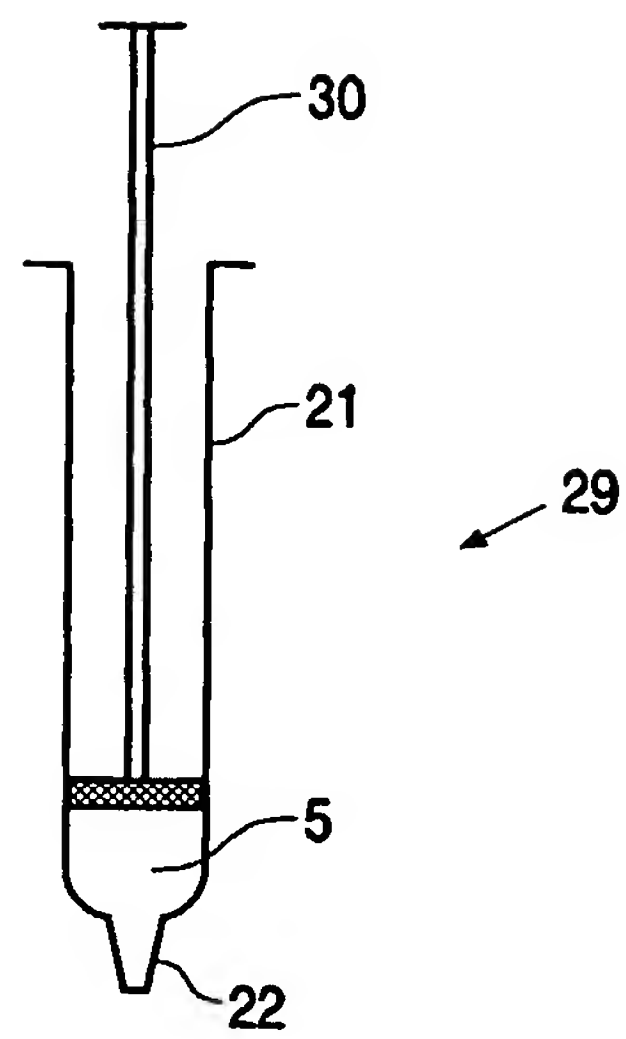


FIG. 10

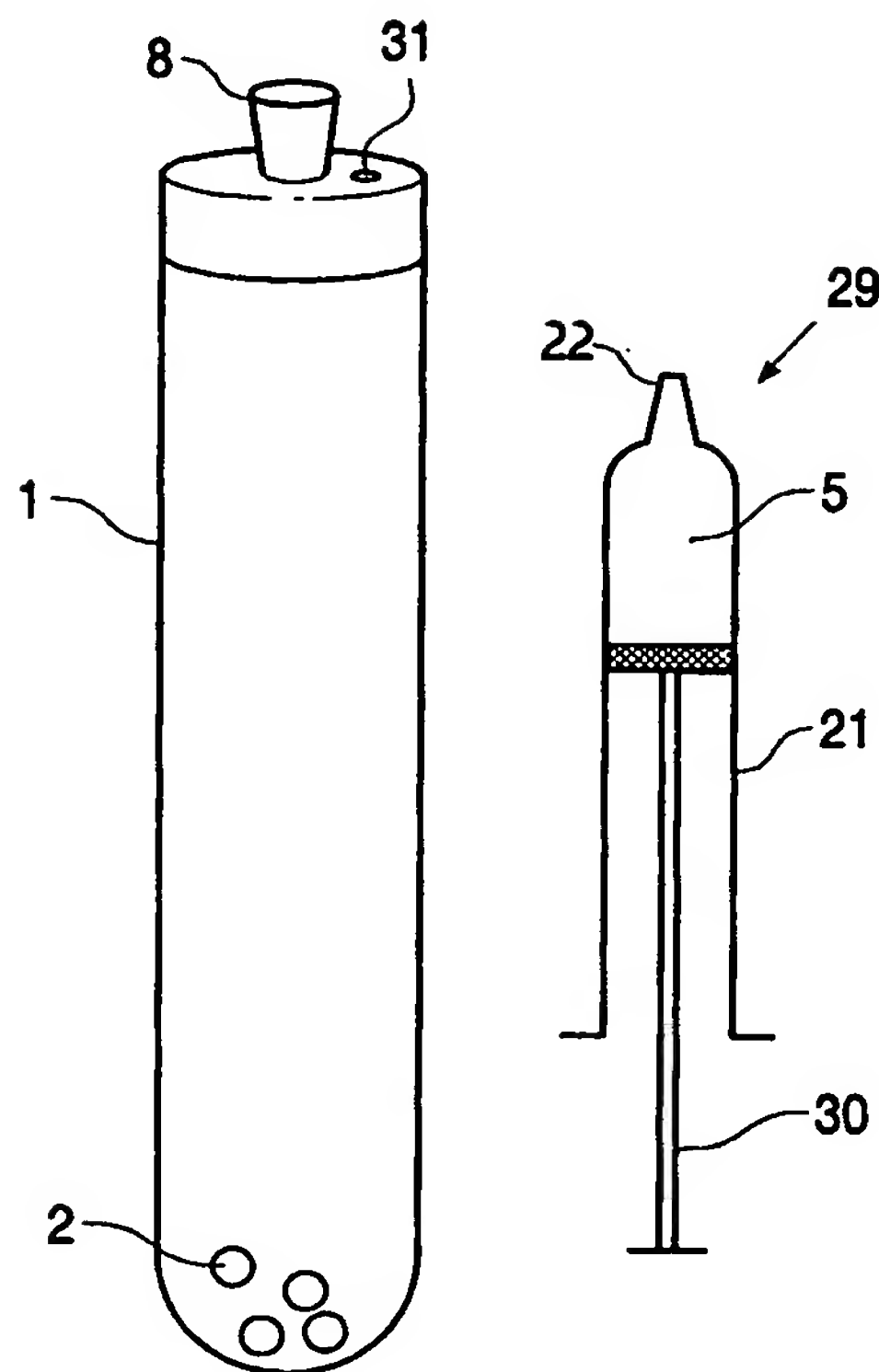


FIG. 11

Figure 12. Strategies followed in the given examples

Figure 12.1 *Ex vivo* monitoring of immune response against tetanus toxoid.

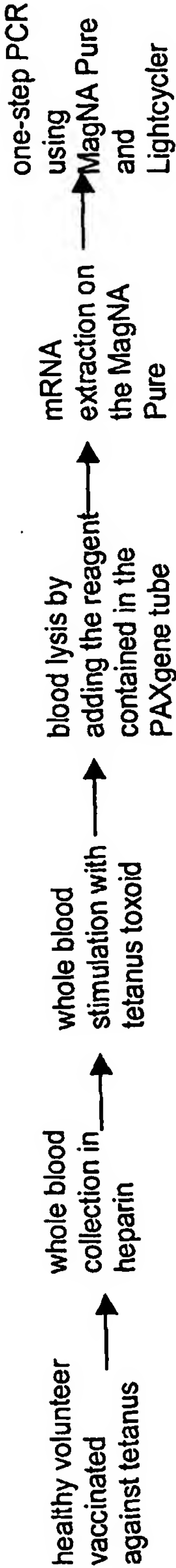


Figure 12.2 Strategy followed in example 3.

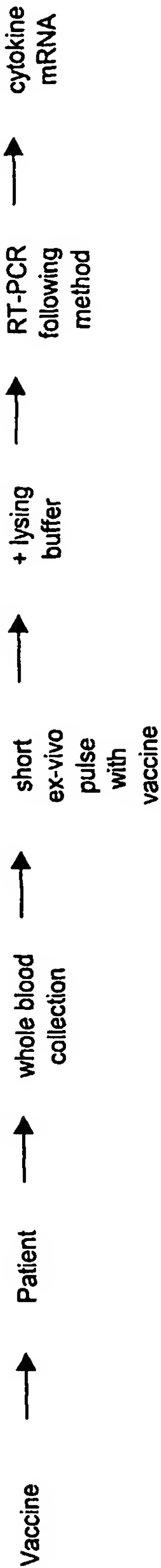


Figure 12.3 Strategy followed in example 4

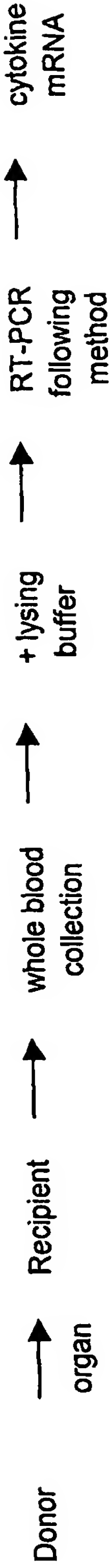
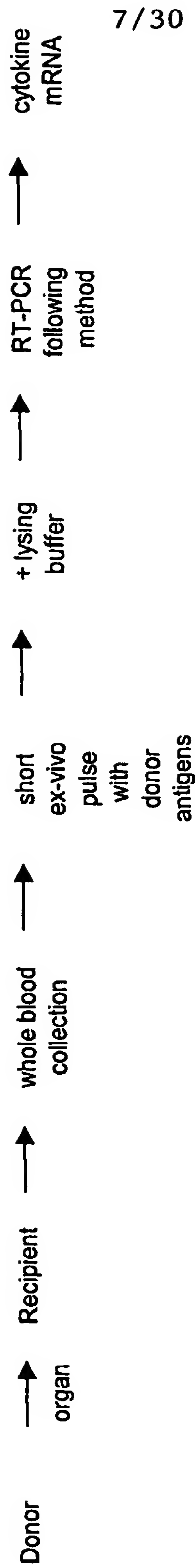


Figure 12.4 Strategy followed in example 5.



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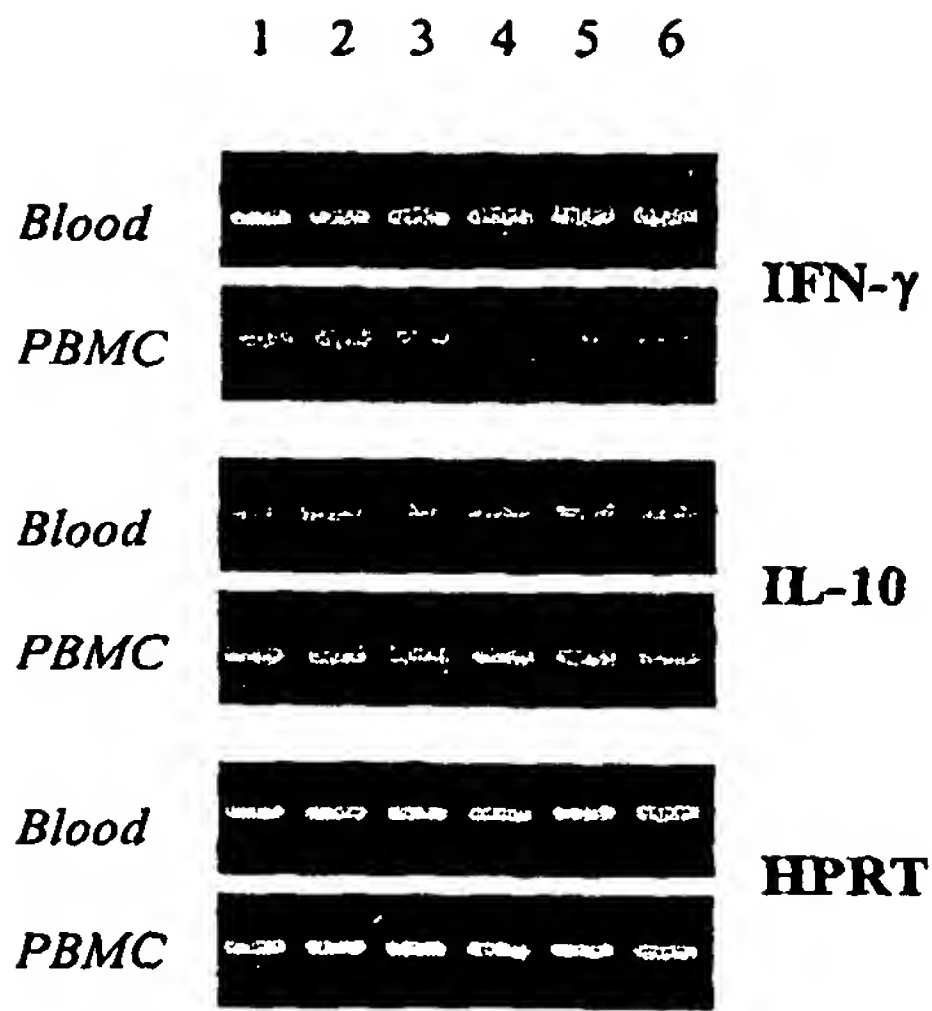


Figure 13.1



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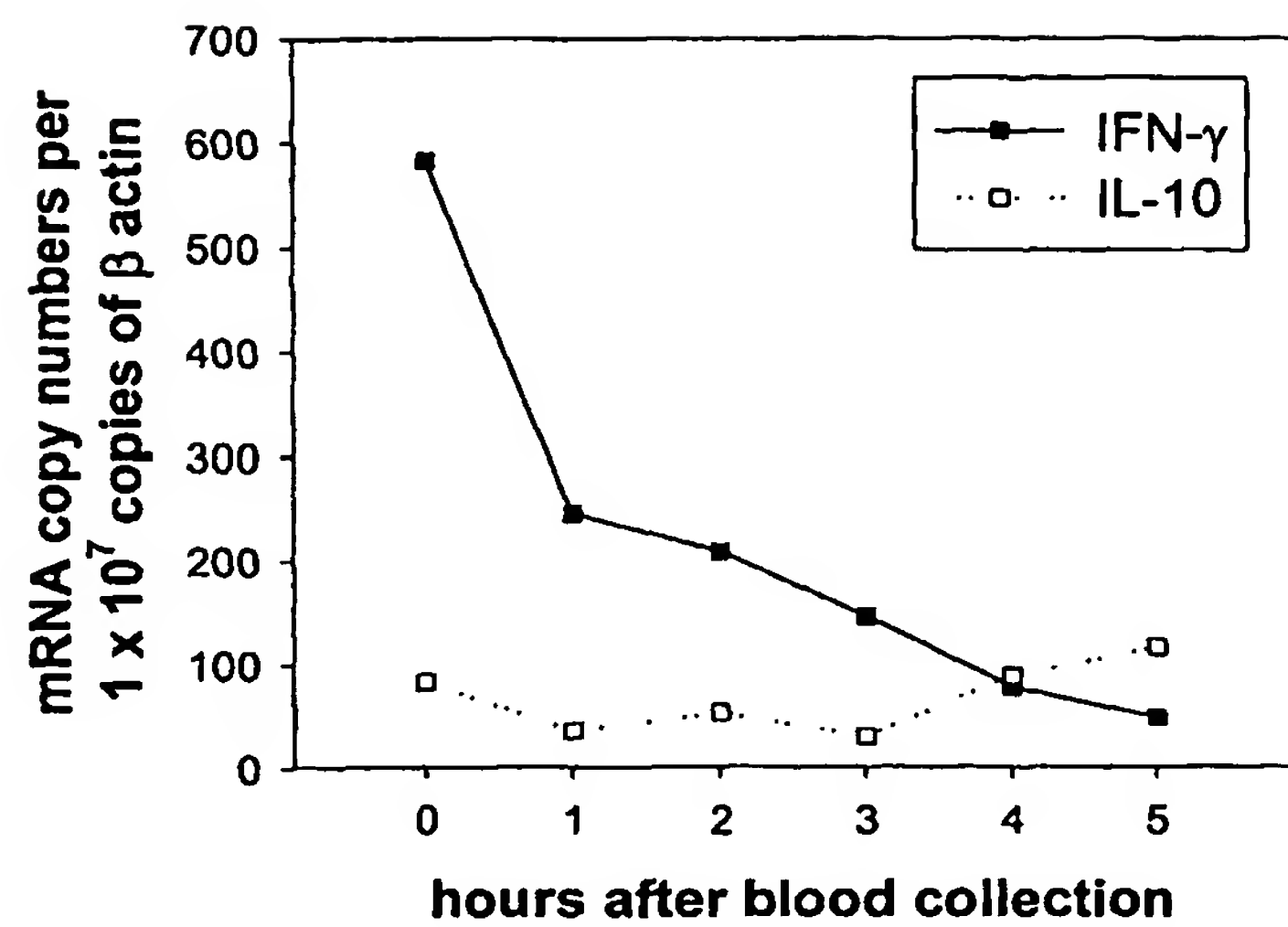


Figure 13.2

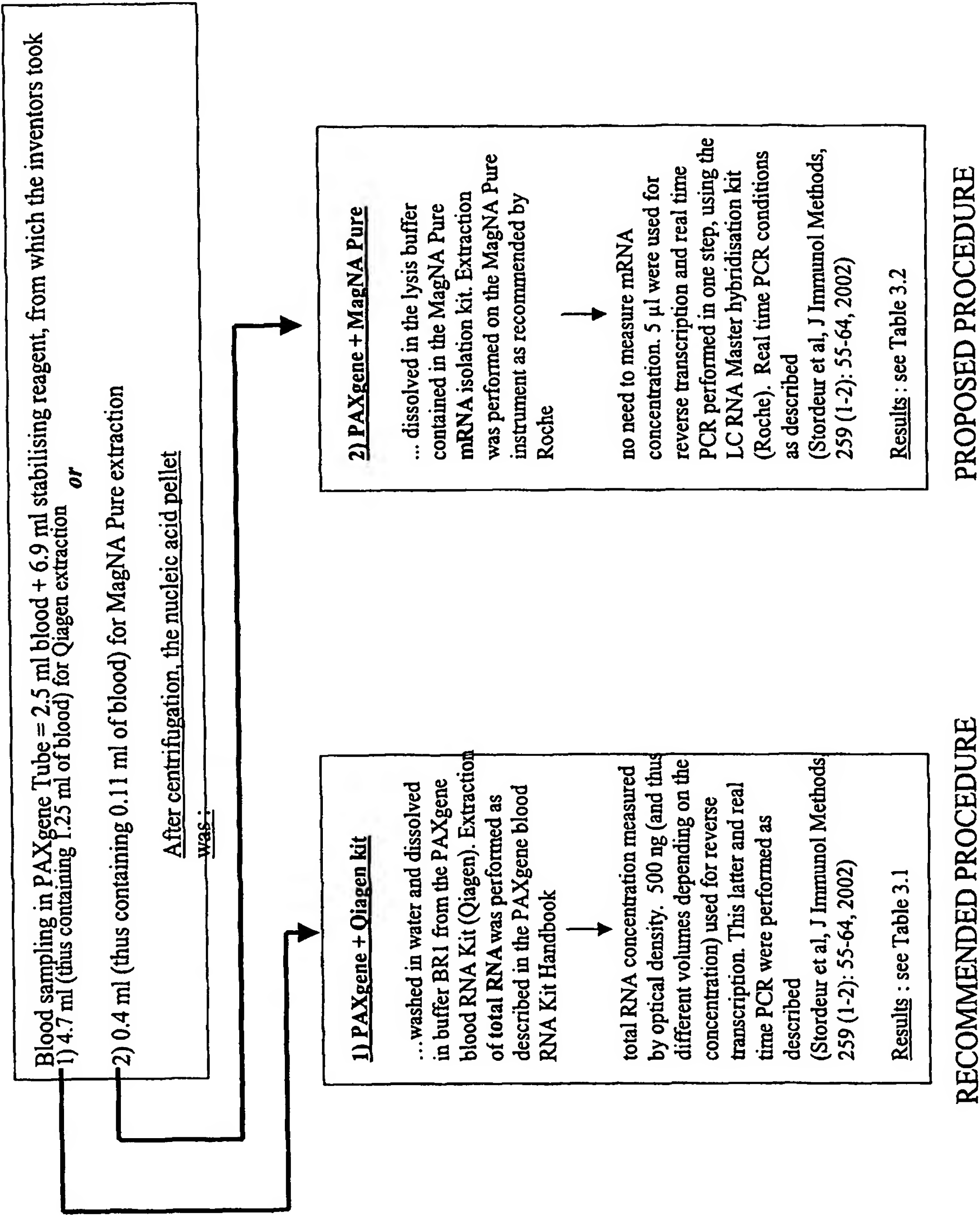


Figure 14

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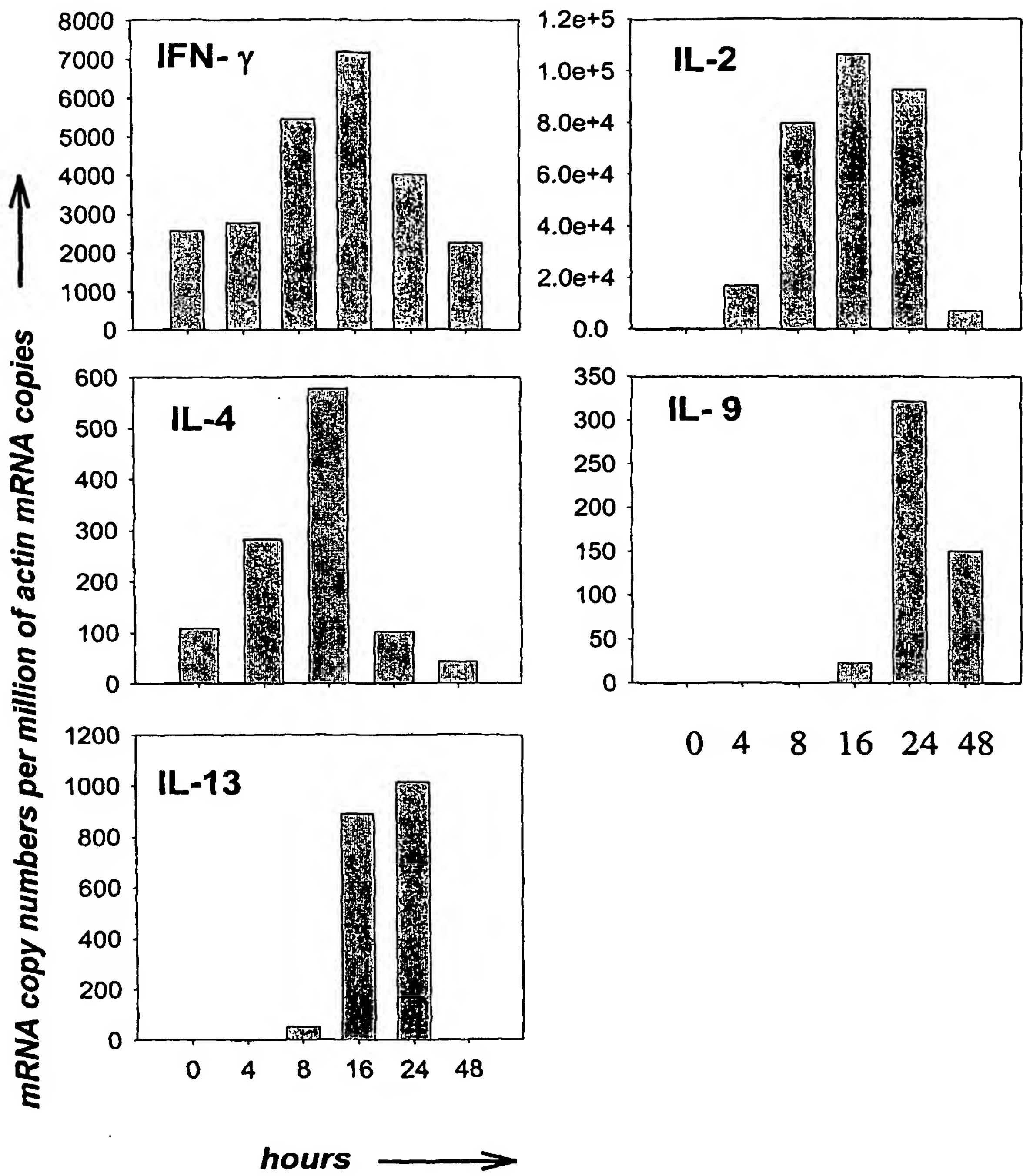


Figure 15

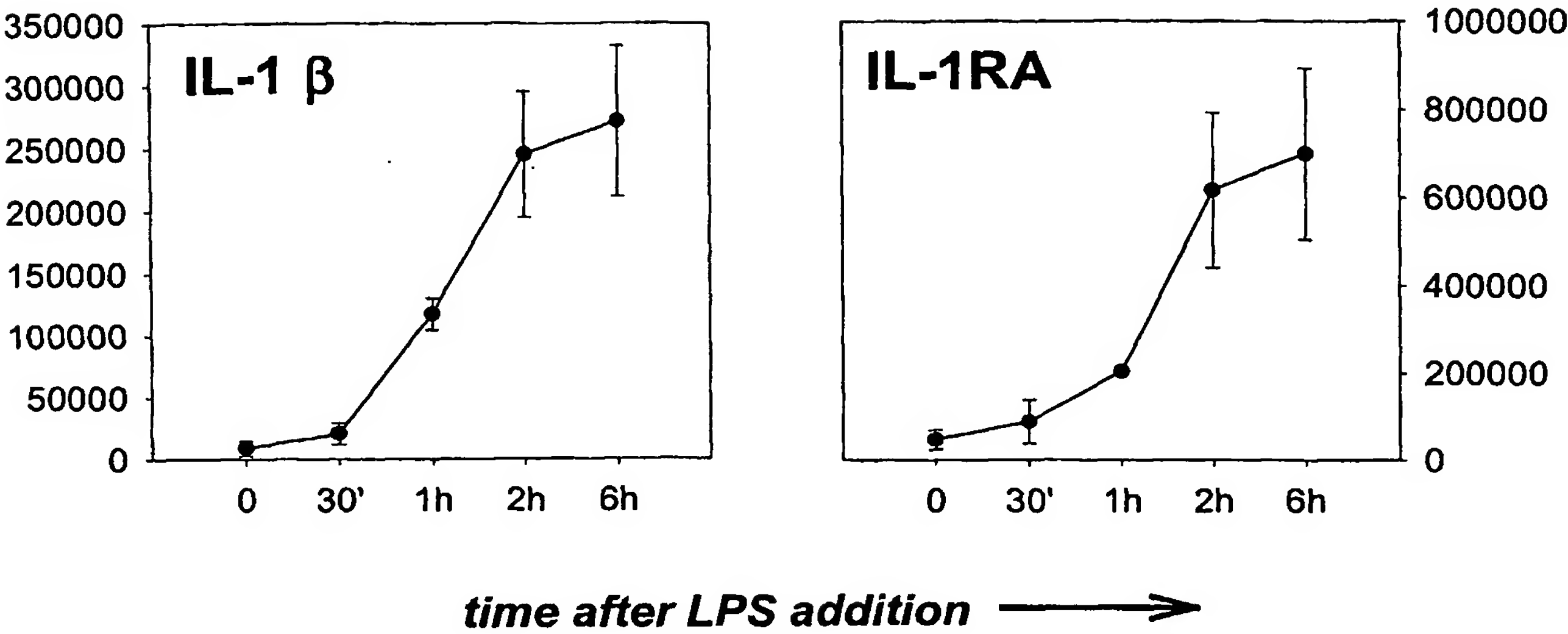


Figure 16

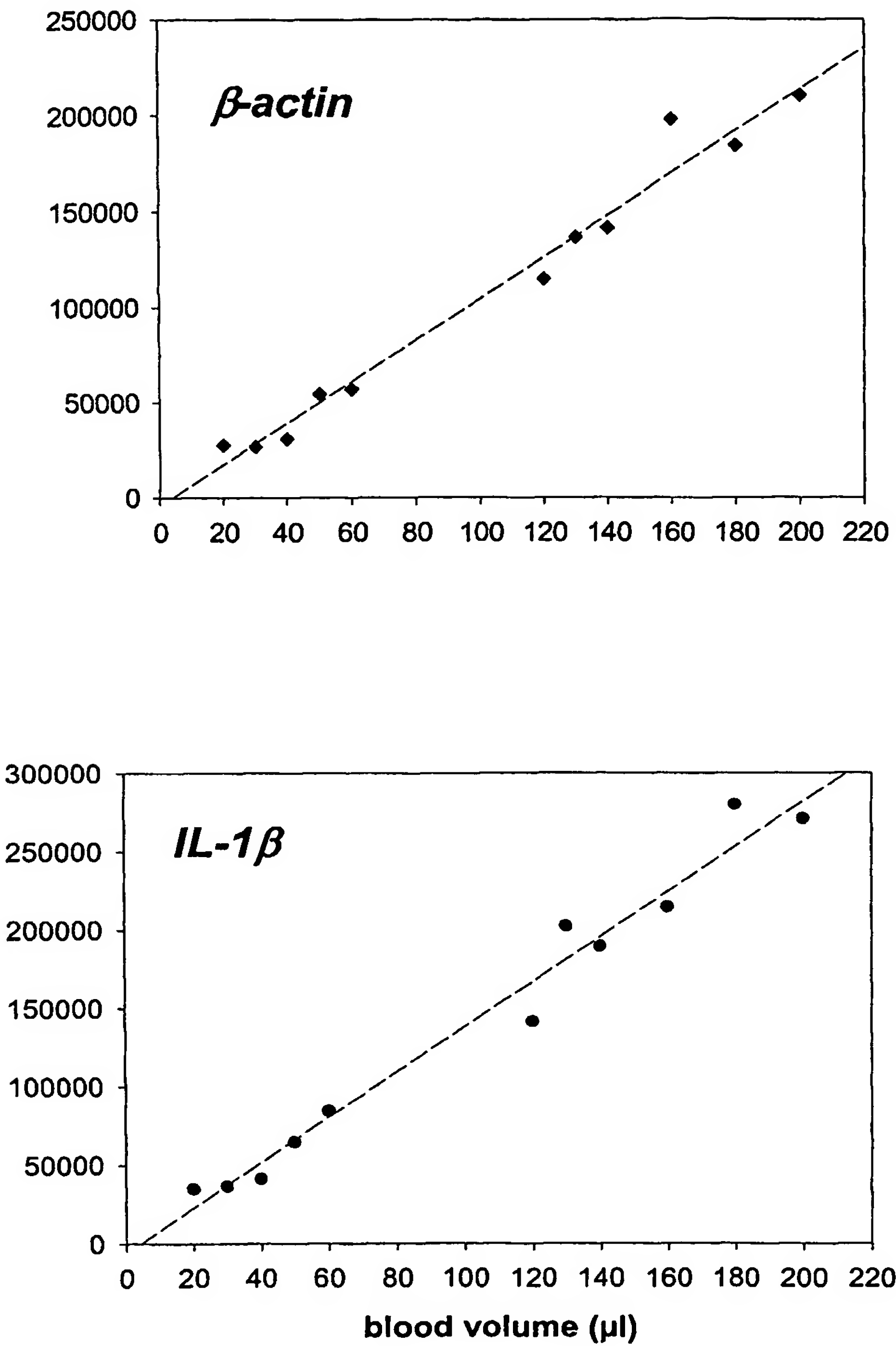


Figure 17

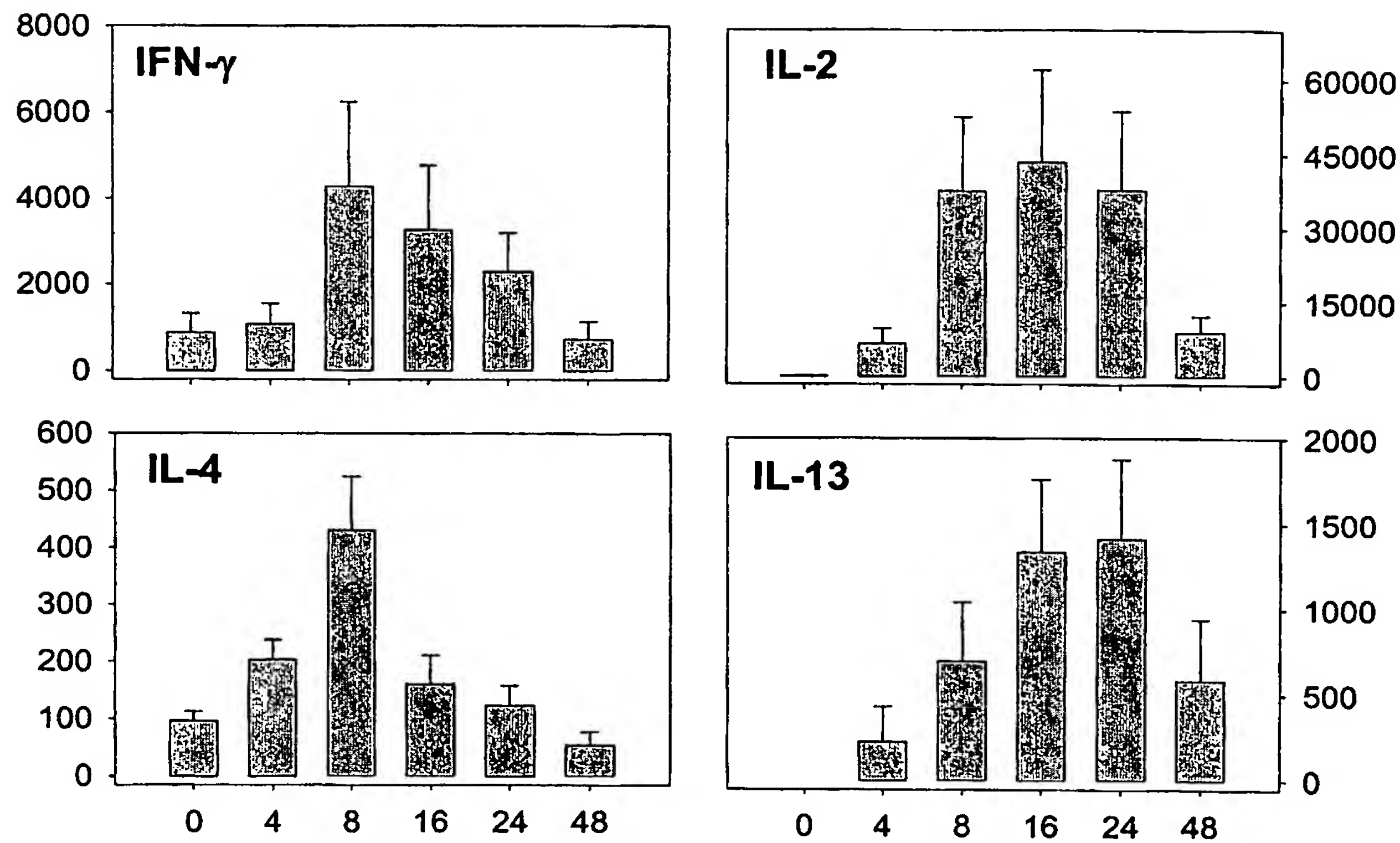


Figure 18

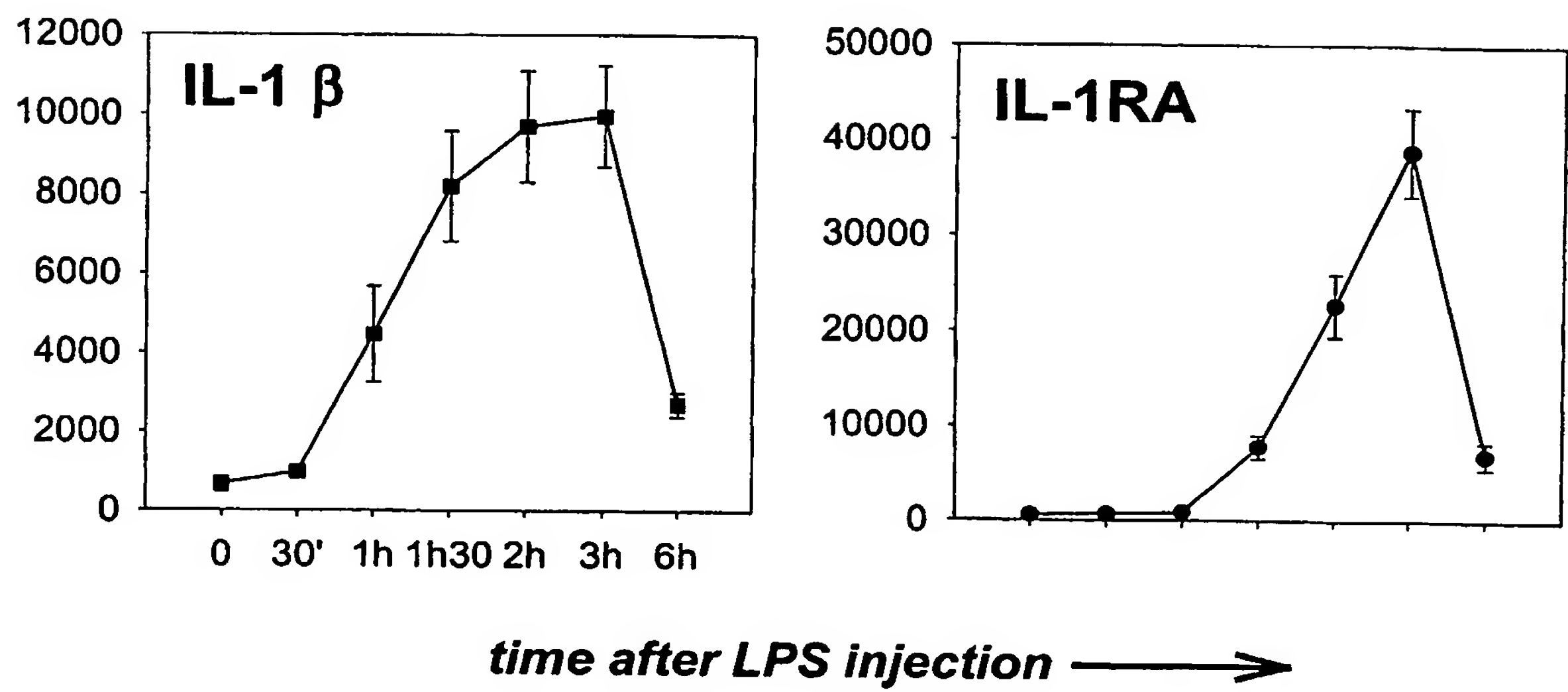


Figure 19

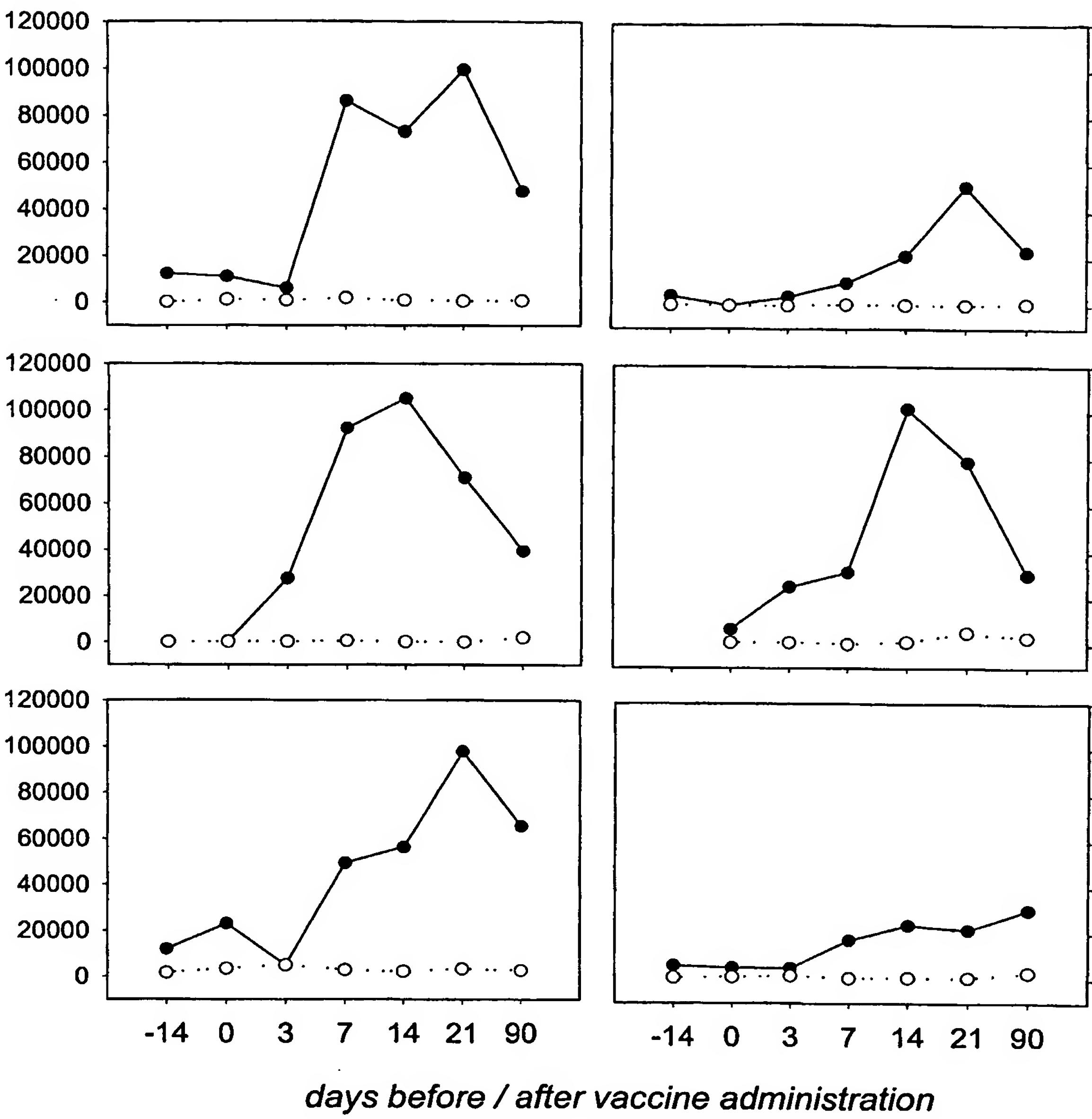


Figure 20



### Procedure to analyse blood cytokine mRNA expression

- **Whole blood** sample incubation in the presence the tested potential immuno-modulator (antigen, allergen, cell, xenobiotic,...)
- Blood cells lysis and **mRNA stabilisation** using the reagent contained in the *PAXgene* tube
- **Automated** mRNA extraction and preparation of the reaction mixture for one-step RT-PCR using an automated device (*Magna Pure instrument – Roche Diagnostics*)
- Quantification of cytokine mRNA levels by **real-time PCR** using the *Lightcycler instrument (Roche Diagnostics)*

Figure 21

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**Automated mRNA extraction and reagent mix preparation  
on the MagNA Pure : direct correlation between starting  
cell number and found copy number**

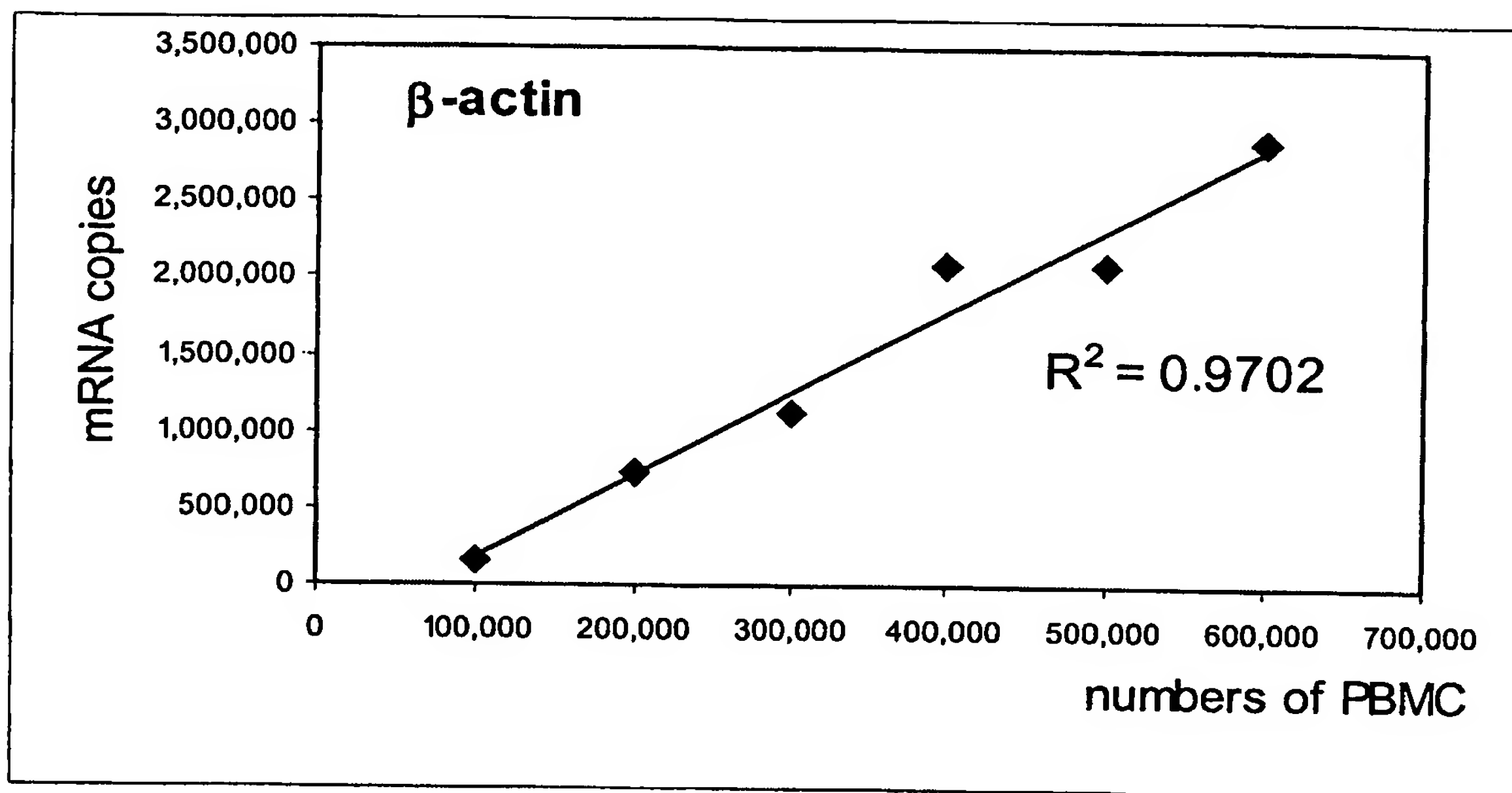


Figure 22

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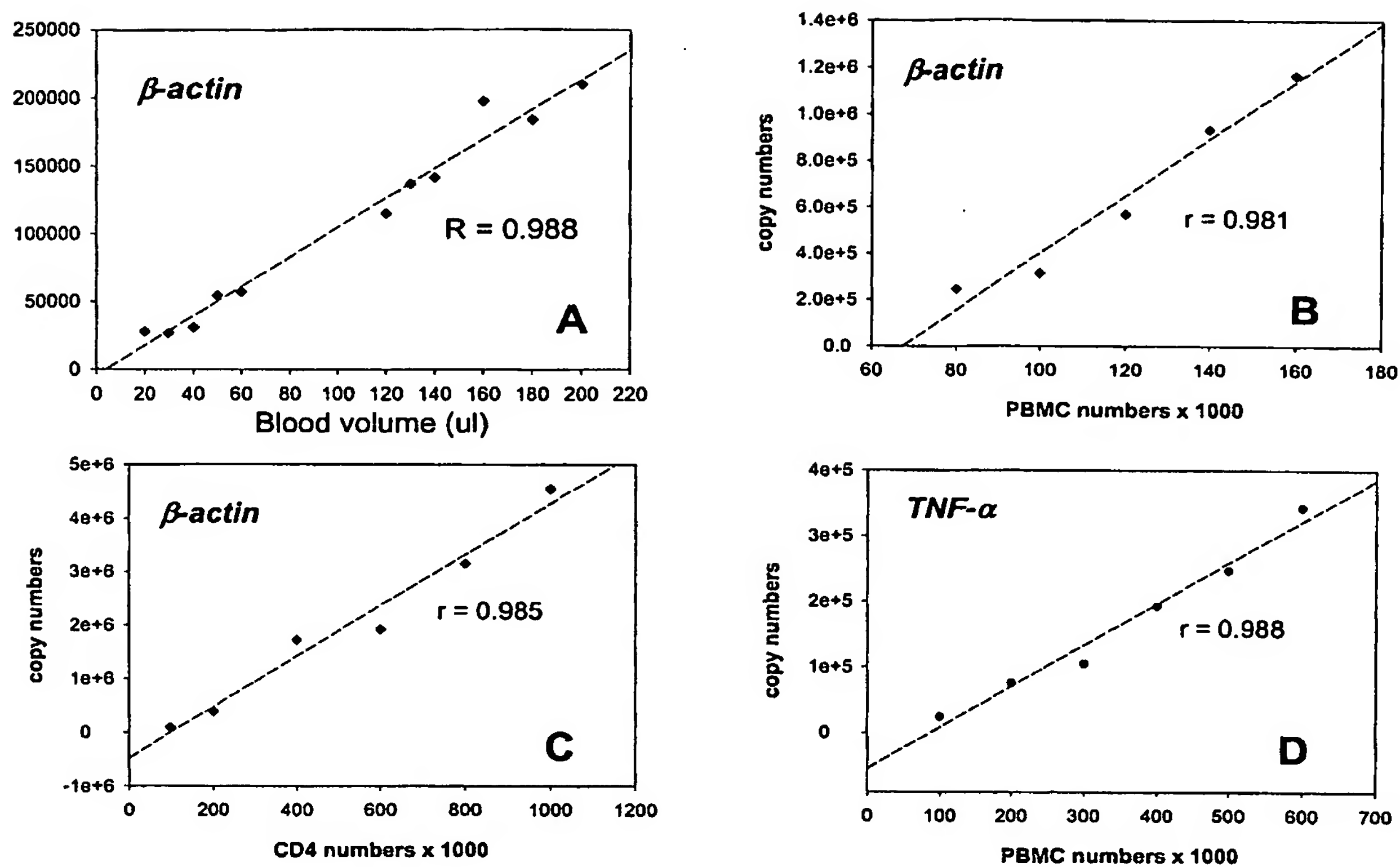


Figure 23

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### **Immune monitoring in cancer immunotherapy**

- 7/99: melanoma of the right scapula. Excision
- 8/01: SC M+ arm, back, abdomen, right testis
- 4/02: right orchidectomy
- 4/02: pre-vaccine check-up
- 5/02: initiation of the vaccine program

**Figure 24**

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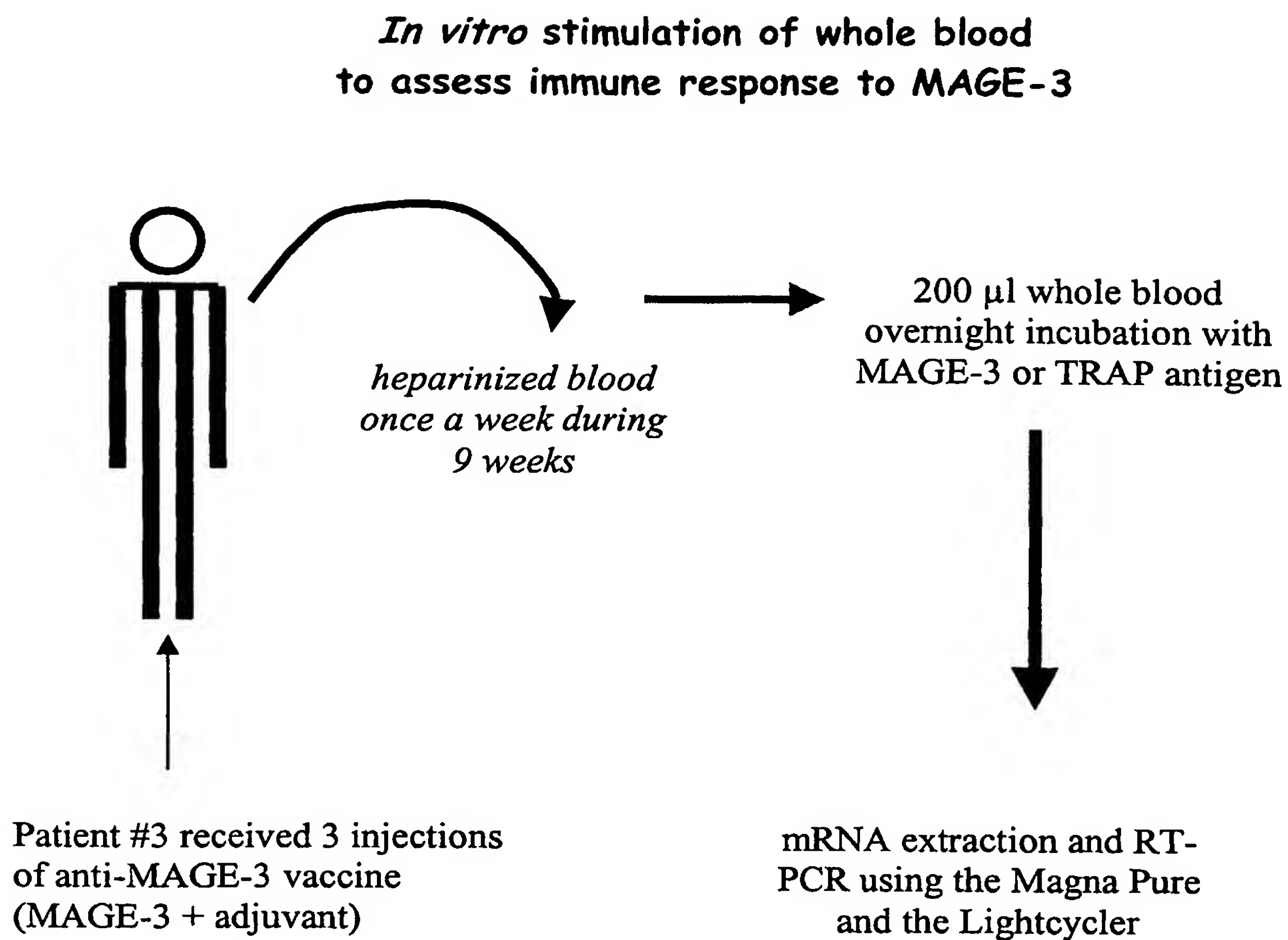


Figure 25

IL-2 mRNA in whole blood following MAGE-3  
vaccination in Patient #3

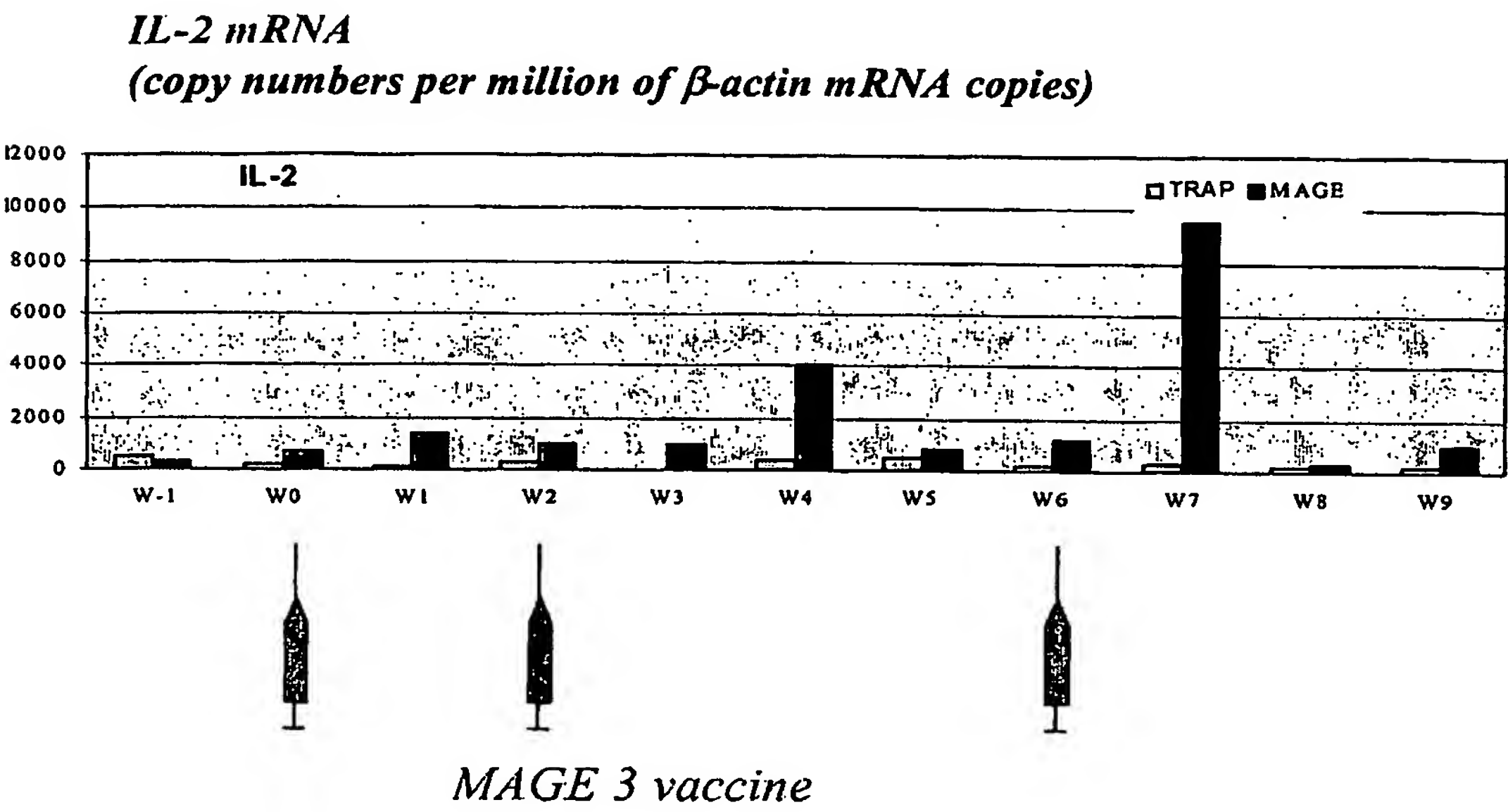
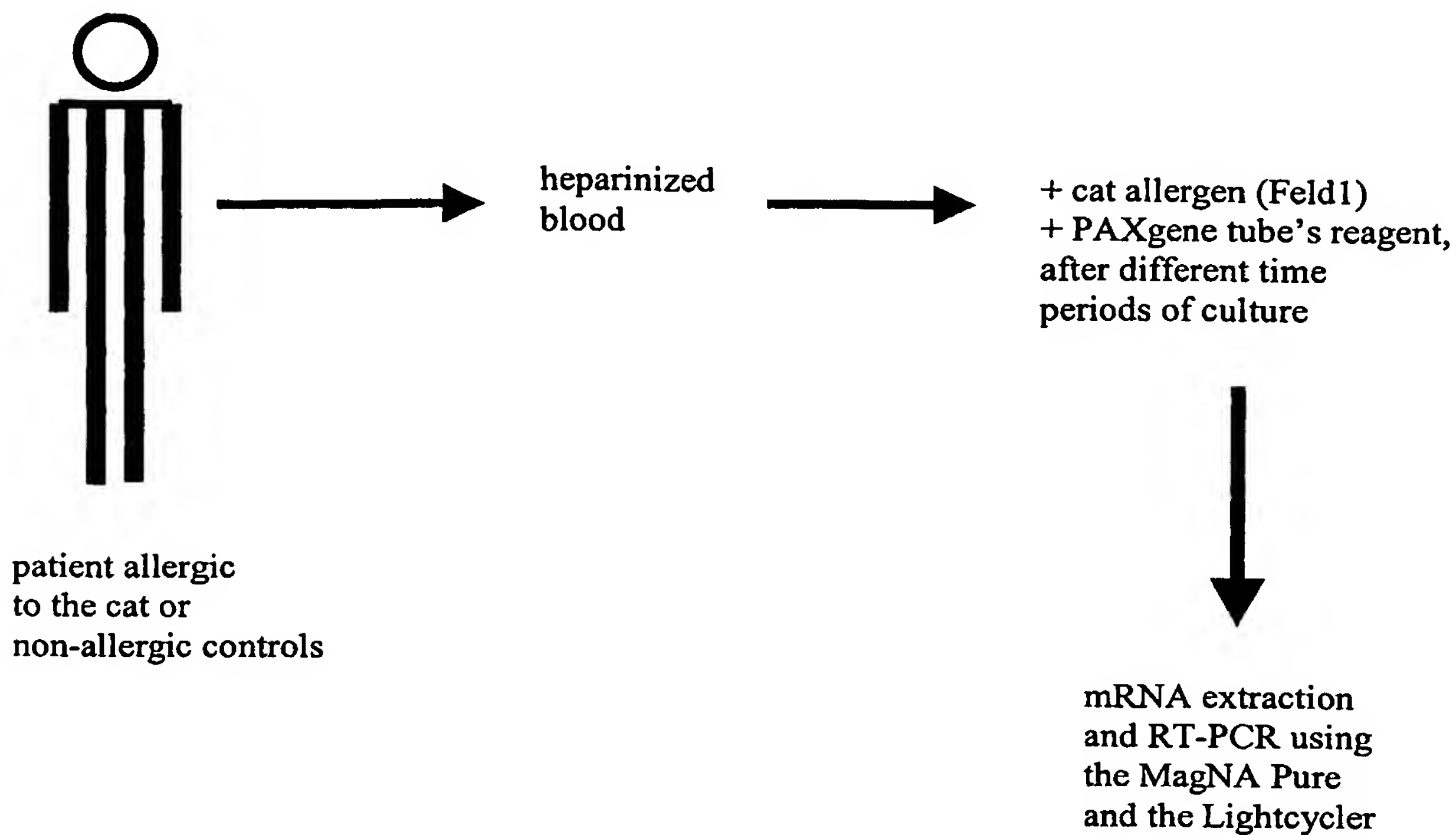


Figure 26

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***In vitro* stimulation of whole blood : evaluation of  
immune response to allergen**



**Figure 27**

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*In vitro* stimulation of whole blood : evaluation of  
immune response to Feld1

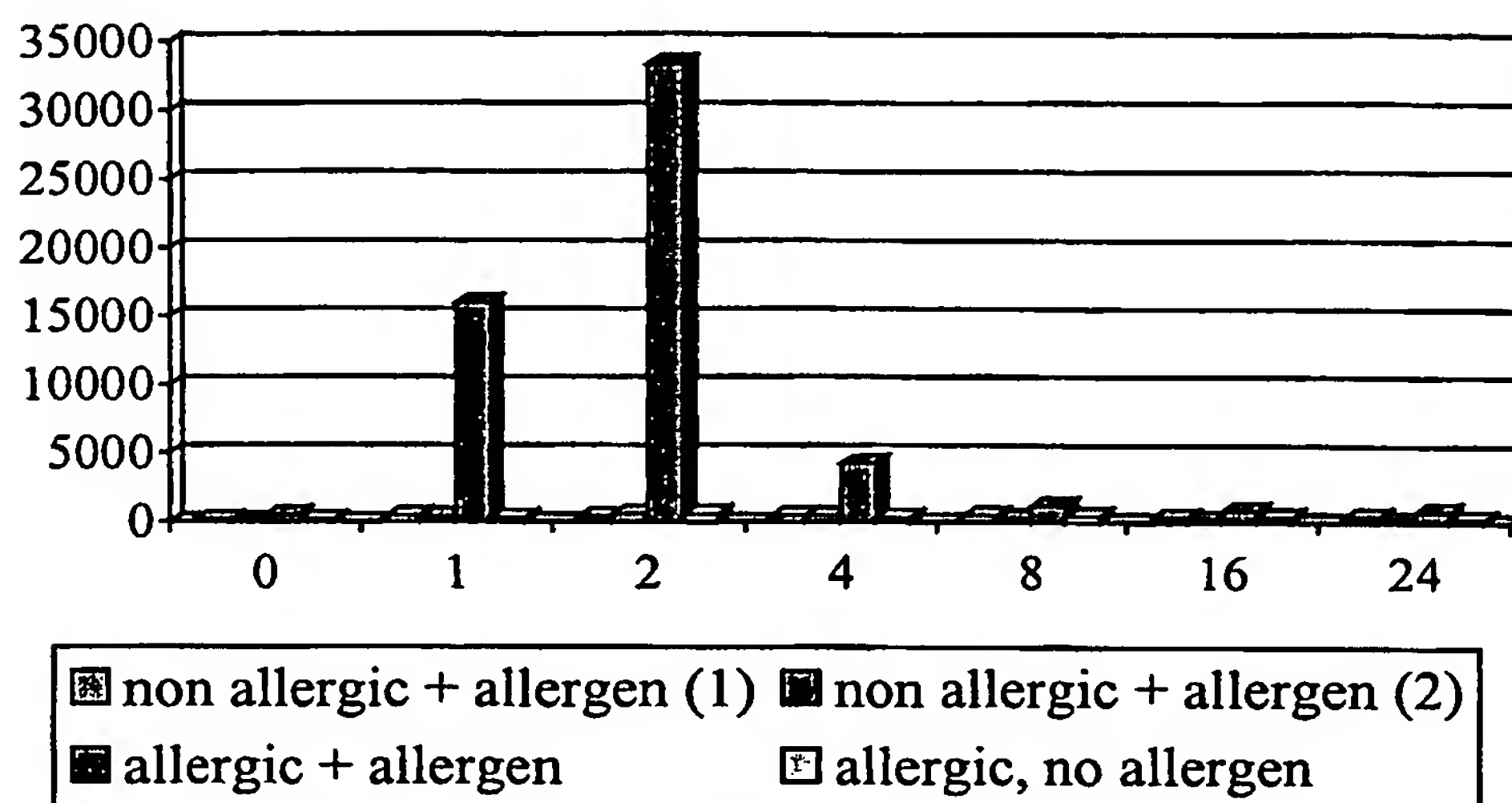


Figure 28



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*In vitro* stimulation of whole blood :  
dose-response to Feld1

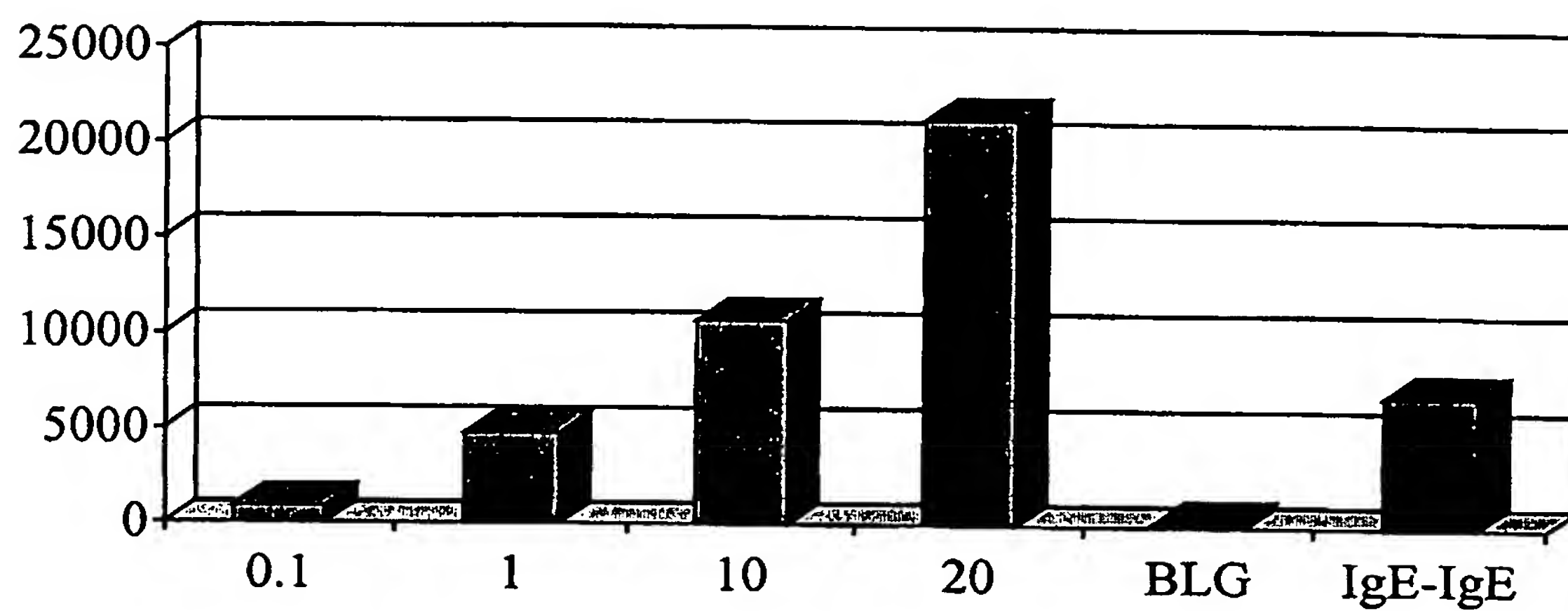


Figure 29

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IL-4 mRNA levels after whole blood stimulation with Feld1  
are higher in allergic patients compared to healthy  
controls

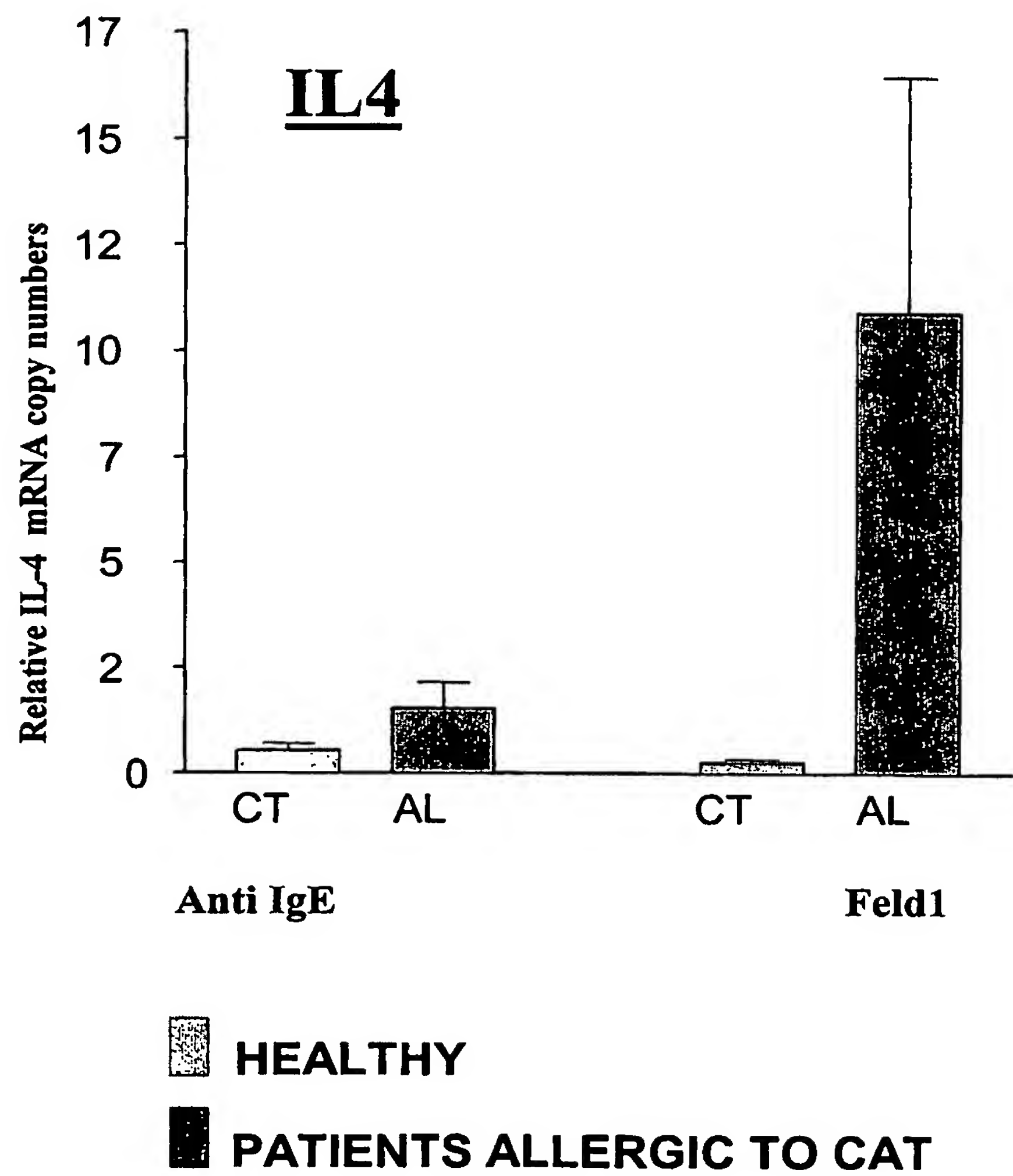


Figure 30

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***In vitro* stimulation of whole blood : assessment  
of T cell response to GAD65**

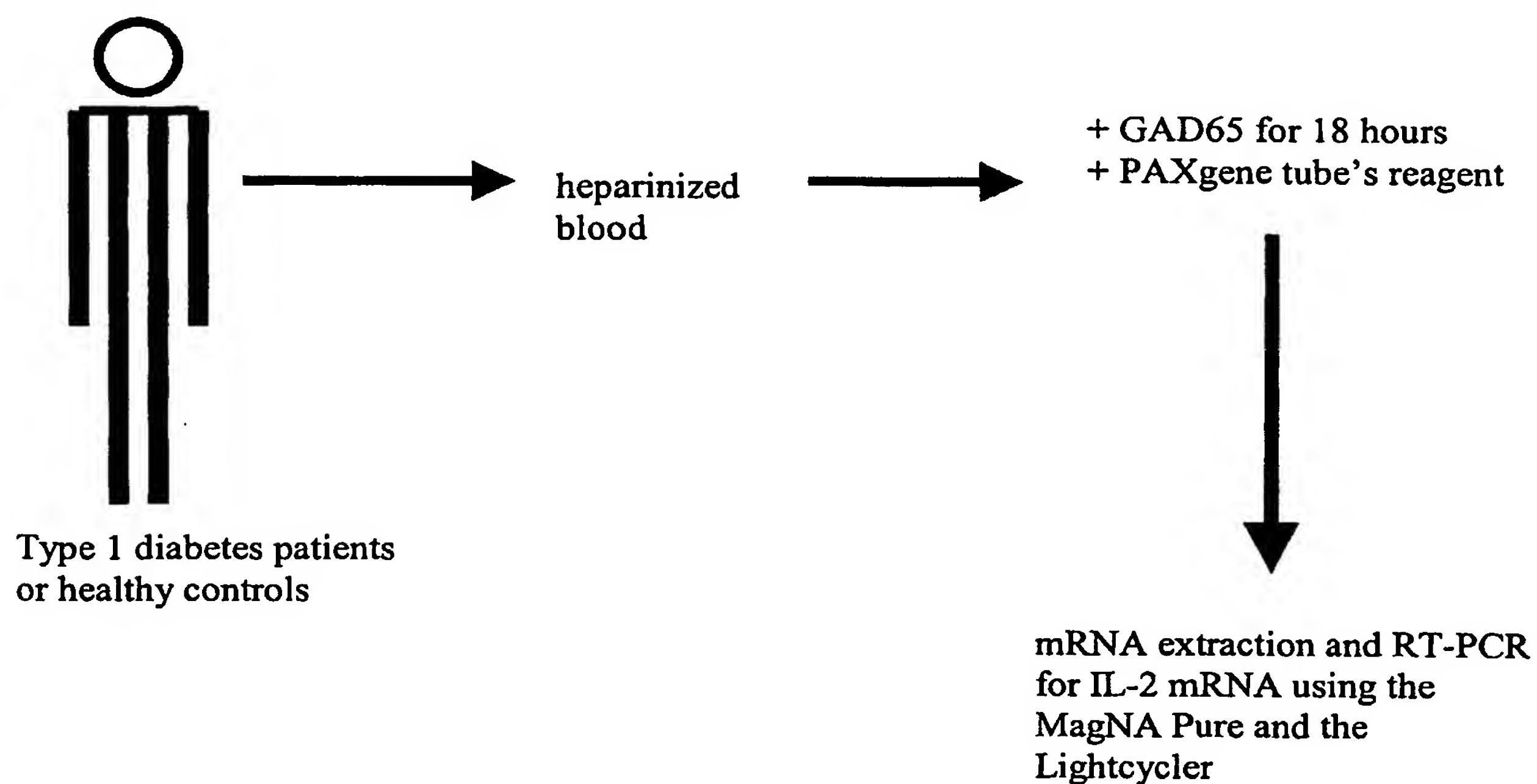


Figure 31

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*In vitro* stimulation of whole  
blood : assessment of T cell  
response to GAD65

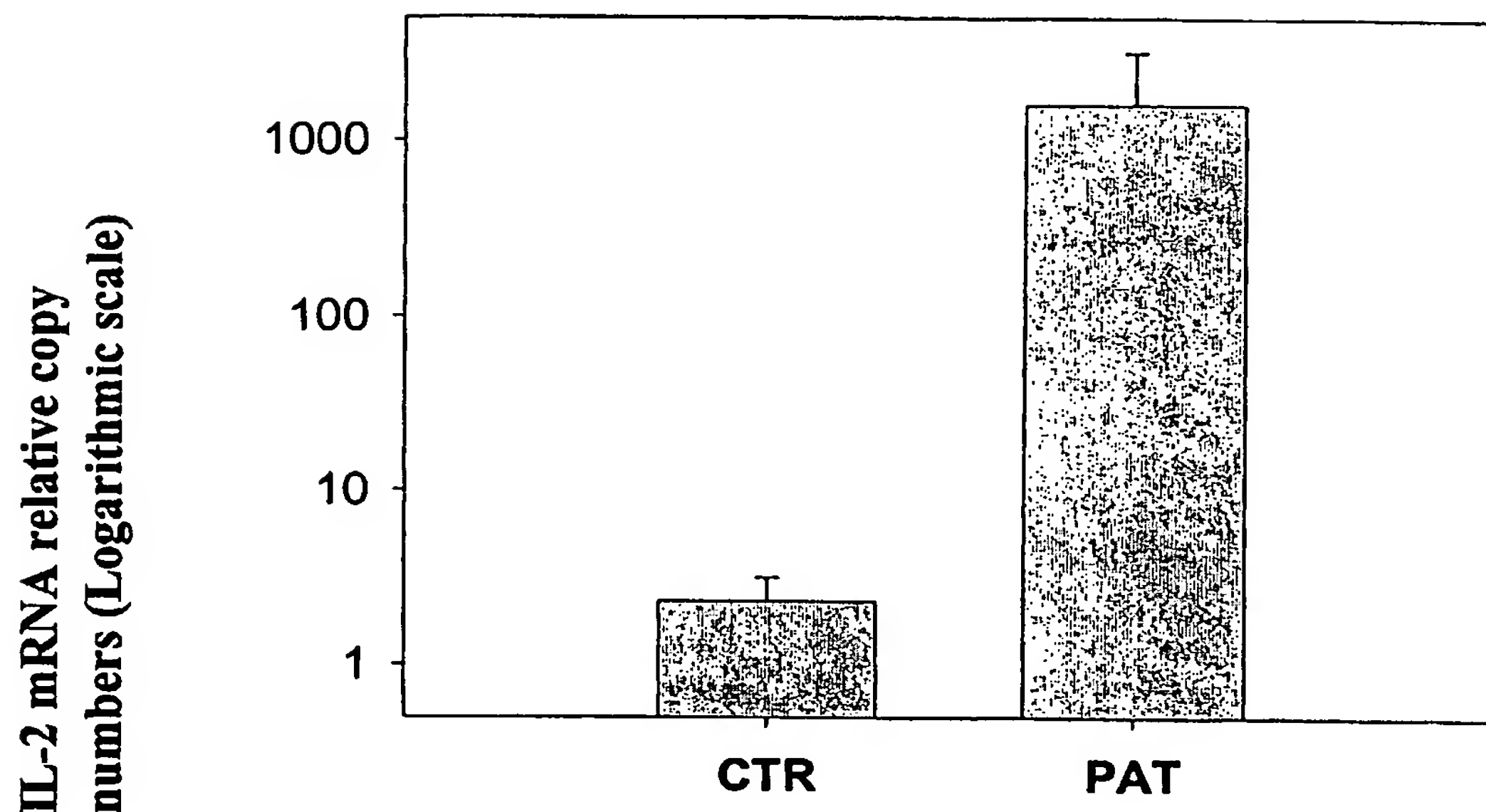


Figure 32

Monitoring of alloreactive immune response :  
quantification of IL-2 mRNA in a whole blood +  
dendritic cells system

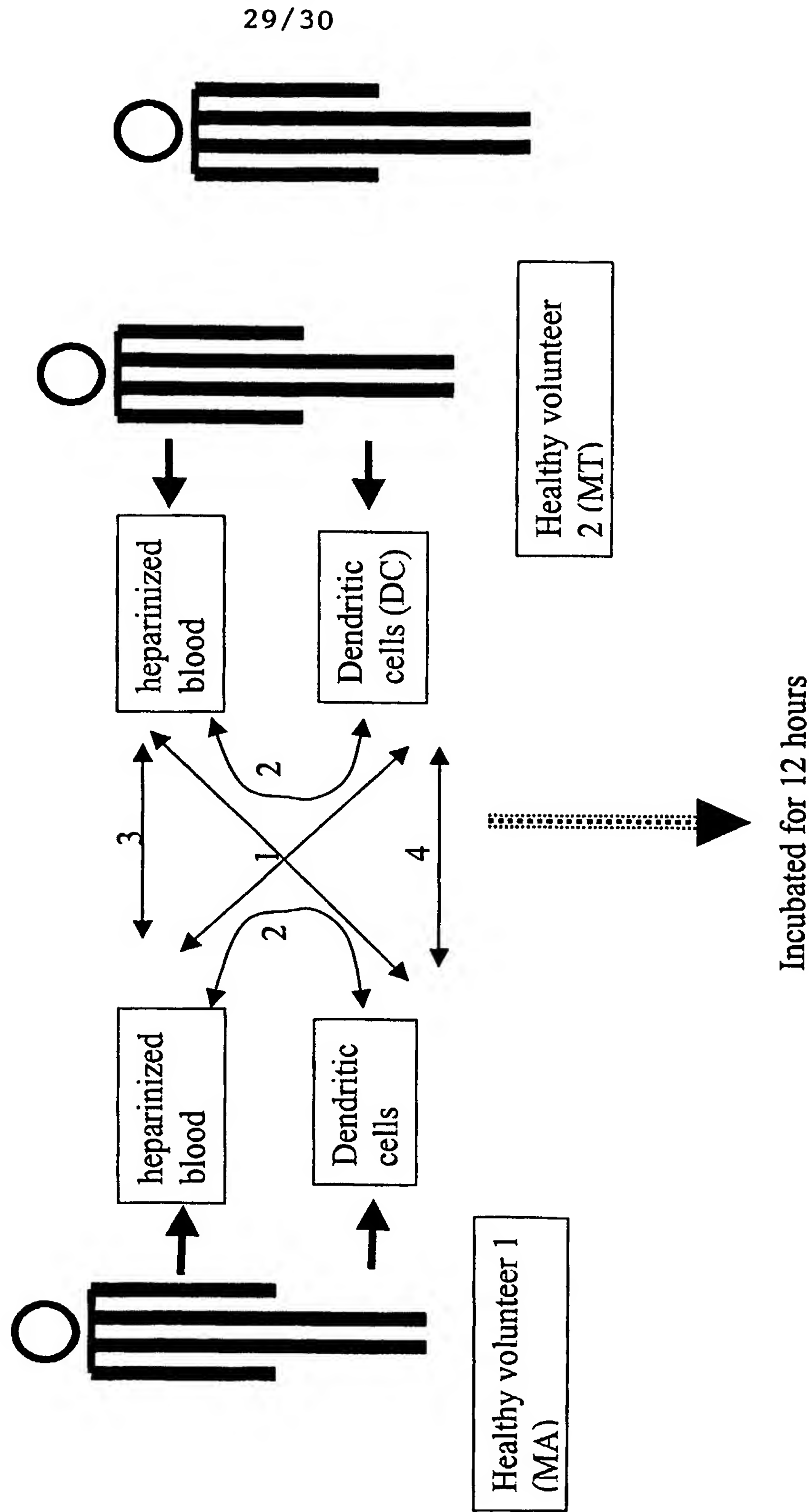


Figure 33

Monitoring of alloreactive immune response :  
quantification of IL-2 mRNA in a whole blood +  
dendritic cells system

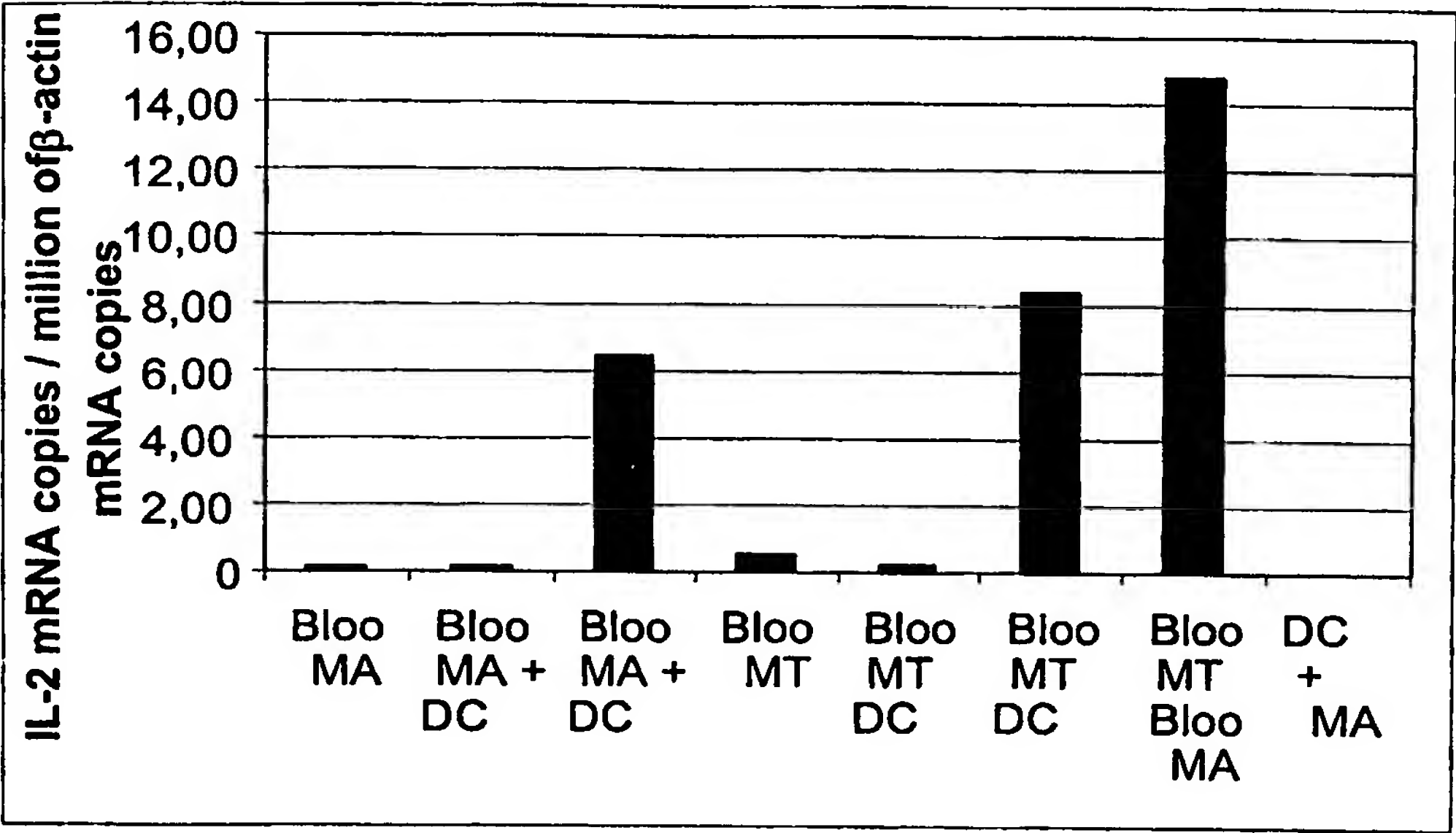


Figure 34